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Proposed Registration Decision PRD2012-17

Aureobasidium pullulans strain DSM 14940 and *Aureobasidium pullulans* strain DSM 14941

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Overview

Proposed Registration Decision for *Aureobasidium pullulans* strain DSM 14940 and *Aureobasidium pullulans* strain DSM 14941

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Aureobasidium pullulans* DSM 14940, *Aureobasidium pullulans* DSM 14941 and Blossom Protect, containing the technical grade active ingredients *Aureobasidium pullulans* strain DSM 14940 and strain DSM 14941, to control fire blight in pome fruits and suppression of fire blight in woody Rosaceae ornamentals.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of *Aureobasidium pullulans* DSM 14940, *Aureobasidium pullulans* DSM 14941 and Blossom Protect.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*.

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact."

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment (for example, those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the PMRA's website at healthcanada.gc.ca/pmra.

Before making a final registration decision on *Aureobasidium pullulans* strain DSM 14940 and *Aureobasidium pullulans* strain DSM 14941, the PMRA will consider all comments received from the public in response to this consultation document[3]. The PMRA will then publish a Registration Decision[4] on *Aureobasidium pullulans* strain DSM 14940 and *Aureobasidium pullulans* strain DSM 14941, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What Is *Aureobasidium pullulans* strain DSM 14940 and *Aureobasidium pullulans* strain DSM 14941?

Aureobasidium pullulans strains DSM 14940 and DSM 14941 are the active ingredients in the end-use product (EP), Blossom Protect. These strains of fungi are used as microbial pest control agents (MPCA) against fire blight caused by *Erwinia amylovora* in pome fruits and woody Rosaceae ornamentals. Both strains DSM 14940 and DSM 14941 of *A. pullulans* were originally isolated from apple leaves of an untreated apple plantation in Germany.

The two *A. pullulans* strains are living yeasts that compete against the fire blight pathogen for space and nutrients under the low pH of the spray solution provided by the citric acid buffer. The fire blight pathogen is not adapted to grow under a low pH environment.

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3 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

4 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

Health Considerations

Can Approved Uses of *Aureobasidium pullulans* strain DSM 14940 and *Aureobasidium pullulans* strain DSM 14941 Affect Human Health?

Aureobasidium pullulans strain DSM 14940 and _A. pullulans_ strain DSM 14941 are unlikely to affect your health when Blossom Protect is used according to the label directions.

People could be exposed to *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 when handling and applying Blossom Protect. When assessing health risks, several key factors are considered:

- the microorganism's biological properties (for example, production of toxic byproducts);
- reports of any adverse incidents;
- its potential to cause disease or toxicity as determined in toxicological studies; and
- the level to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns. When spores of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 were tested on laboratory animals, there were no signs that it caused any significant toxicity or disease.

Residues in Water and Food

Dietary risks from food and water are not of concern

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the Pest Control Products Act (PCPA) for the purposes of the adulteration provision of the Food and Drugs Act (FDA). Health Canada sets science-based MRLs to ensure that the food Canadians eat is safe.

Aureobasidium pullulans is a ubiquitous yeast-like fungus that is commonly found in the phyllosphere. The level of *A. pullulans* on pome fruit is not expected to significantly increase due to the application of Blossom Protect since it is found in the phyllosphere at concentrations comparable to the application rate. Any increase in the population of *A. pullulans* that may occur is expected to return to natural levels at the time of harvest since application of Blossom Protect is to be made during bloom. Furthermore, when *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 were administered orally to rats, no signs of toxicity or disease were observed, and no metabolites of toxicological significance have been shown to be produced by this or other strains of *A. pullulans*. Therefore the establishment of a MRL is not required for

A. pullulans strain DSM 14940 or *A. pullulans* strain DSM 14941. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary risks are minimal to non-existent.

Occupational Risks From Handling Blossom Protect

Occupational risks are not of concern when Blossom Protect is used according to label directions, which include protective measures

Growers handling Blossom Protect can come into direct contact with *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 on the skin, in the eyes or by inhalation. For this reason, the product labels specify that growers exposed to Blossom Protect must wear waterproof gloves, coveralls, a NIOSH-approved respirator (with any N-95, P-95, R-95 or HE filter for biological products), and shoes plus socks. Eye goggles are not required as the eye irritation studies submitted indicated minimal eye irritation potential.

For the bystander, exposure is expected to be much less than that of handlers and mixer/loaders and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happen When *Aureobasidium pullulans* strain DSM 14940 and *Aureobasidium pullulans* strain DSM 14941 are Introduced into the Environment?

Environmental risks are not of concern

The active ingredients contained in Blossom Protect, *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941, are individual isolates of the species *A. pullulans* which is a ubiquitous yeast-like organism. Following application, levels of *A. pullulans* DSM strain 14940 and *A. pullulans* strain DSM 14941 in the environment are comparable to observable levels of naturally occurring *A. pullulans*. Since naturally occurring background levels of *A. pullulans* vary, levels may temporarily increase after application, however, it is expected that the population of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 would return to the site specific natural background levels over the course of the growing season.

Studies were conducted to determine the effects of *A. pullulans* DSM 14941 on birds and of Blossom Protect (containing *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941) on fish, bees, terrestrial and aquatic arthropods, and aquatic plants. These studies showed that *A. pullulans* DSM 14941 was not toxic or pathogenic to birds, and that Blossom Protect was not toxic or pathogenic to fish, bees, terrestrial and aquatic arthropods, or aquatic plants.

Although terrestrial non-arthropod invertebrate, plant, and aquatic non-arthropod invertebrate, and microorganism toxicity/pathogenicity testing were not assessed in the review, adequate information was available to determine that significant adverse effects to these non-target organisms are not expected. The level of exposure to these non-target organisms from the application of Blossom Protect are expected to be comparable to exposures that could occur from natural populations of *A. pullulans*. Furthermore, a search of published scientific literature did not result in any reports of adverse effects to non-target organism from *A. pullulans*.

Value Considerations

What Is the Value of Blossom Protect?

***Aureobasidium pullulans* strain DSM 14940 and *Aureobasidium pullulans* strain DSM 14941, the active ingredients in Blossom Protect, control fire blight in pome fruits (bearing and non-bearing) and suppress fire blight in woody Rosaceae ornamentals.**

Blossom Protect, containing 2.5 x 10^9 CFU/g *A. pullulans* strain DSM 14940 and 2.5 x 10^9 CFU/g *A. pullulans* strain DSM 14941, is a product formulated as a crown treatment against fire blight in pome fruits (bearing and non-bearing) and in woody Rosaceae ornamentals. A citric acid buffer is included in the formulation to ensure a consistent low pH value, which is essential for the initial growth of the *A. pullulans* strains.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the label of Blossom Protect to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

In individuals exposed to large quantities of Blossom Protect, respiratory and dermal sensitivity could possibly develop upon repeated exposure to the product since the end-use product has been identified as a sensitizer. Therefore, anyone handling or applying Blossom Protect must wear waterproof gloves, coveralls, a NIOSH-approved respirator (with any N-95, P-95, R-95 or HE filter for biological products), and shoes plus socks. Eye goggles are not required as the eye irritation studies submitted indicated minimal eye irritation potential. An additional risk reduction measure is a restricted entry interval for early-entry workers immediately following product application until sprays have dried. Workers may re-enter before sprays have dried if wearing appropriate personal protective equipment (PPE), including water-proof gloves, long-sleeved shirt, long pants, and shoes plus socks.

Environment

The end-use product label will include environmental precaution statements that prevent the contamination of aquatic systems from the use of Blossom Protect.

Next Steps

Before making a final registration decision on *Aureobasidium pullulans* strain DSM 14940 and *Aureobasidium pullulans* strain DSM 14941, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Aureobasidium pullulans* strain DSM 14940 and *Aureobasidium pullulans* strain DSM 14941 (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Aureobasidium pullulans strain DSM 14940 and *Aureobasidium pullulans* strain DSM 14941

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Aureobasidium pullulans* strain DSM 14940 and *Aureobasidium pullulans* strain DSM 14941
Function	To control fire blight caused by *Erwinia amylovora* on pome fruit and on woody roseaceous ornamentals
Binomial name	*Aureobasidium pullulans* strain DSM 14940 and *Aureobasidium pullulans* strain DSM 14941
Taxonomic designation[1]	
Kingdom	Fungi
Phylum	Ascomycota
Class	Dothideomycetes
Order	Dothideales
Genus	*Aureobasidium*
Species	*pullulans*
Strains	DSM 14940 and DSM 14941
Patent Status information	No patents are held by the applicant in Canada.
Minimum purity of active	Technical grade active ingredients (TGAI): 5.0×10^9 colony forming units (CFU)/g EP: 5.0×10^9 CFU/g (total of both strains DSM 14940 and DSM 14941)
Identity of relevant impurities of toxicological, environmental and/or significance.	The TGAI does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 are not known to produce any potentially toxic secondary metabolites (see Section 3.0).

[1] Taxonomy browser at: http://www.ncbi.nlm.nih.gov/pubmed/

1.2 Physical and Chemical Properties of the Active Ingredients and End-Use Product

Technical Grade Active Ingredient (TGAI) – *Aureobasidium pullulans* strain DSM 14940, *Aureobasidium pullulans* strain DSM 14941 and **End Use Product** (EP) – Blossom Protect

Properties	*Aureobasidium pullulans* strain DSM 14940	*Aureobasidium pullulans* strain DSM 14941	Blossom Protect
Physical state	Granule	Granule	Wettable powder
Colour	Light brown to pink	Light brown to pink	Light brown to pink
Odour	Slightly sweet, bread-like	Slightly sweet, bread-like	Bread-like
pH	n/a	n/a	5.7
Guarantee	5×10^9 CFU/g	5×10^9 CFU/g	5×10^9 CFU/g, both strains
Corrosion Character	None	None	None
Suspendibility	Suspendible	Suspendible	Suspendible
Viscosity	Not applicable	Not applicable	Not applicable

1.3 Directions for Use

Blossom Protect is a biological bactericide containing the yeast *A. pullulans* strain DSM 14940 and strain DSM 14941. This product has two components. Component A contains a citric acid buffer that ensures a consistent pH value on the leaf and blossom surface, and thus provides an optimum condition for the growth of *A. pullulans* strains. Component B contains the two yeast strains, which provide protection from the fire blight pathogen. To prepare the solution, Component A (10.5 kg) is diluted in water and then added to Component B (1.5 kg). The package contents are premeasured for 1000 L spray solution. Spraying solution is to be used within 8 hours of preparation. Blossom Protect can be applied up to four times on pome fruit trees and woody Rosaceae ornamentals at flowering, based on phenology or five times per season based on a disease forecast system.

1.4 Mode of Action

The mode of action of Blossom Protect is the inhibition of growth of fire blight pathogen through pH modification of the growing environment as well as competition for space and nutrients. The low pH of the spray solution provided by the citric acid buffer (Component A) decreases the pH of the plant surfaces which initially provides an optimum environment for the yeasts. The fire blight pathogen, *Erwinia amylovora*, is not adapted to grow under a low pH environment. In addition, the yeasts compete for space and nutrients with *E. amylovora*.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganisms

Aureobasidium pullulans strain DSM 14940 and *A. pullulans* strain DSM 14941 can be identified to the species level through microscopic examination of single spore isolates focusing on standard morphological features along with molecular techniques. The molecular techniques include polymerase chain reaction (PCR) to amplify internal transcribed spacers 1 (ITS1) and 4 (ITS4) of the 18S rDNA gene. Specific PCR primer pairs designed from randomly amplified polymorphic DNA analysis (RAPD) can be used to distinguish the two strains.

2.2 Methods for Establishment of Purity of Seed Stock

Aureobasidium pullulans strain DSM 14940 and *A. pullulans* strain DSM 14941 are officially kept in the German Strain Collection for Microorganisms (DSMZ). Stock solutions are kept frozen under glycerin at -80°C. Every year, viability is checked and new stock solutions are produced.

Practices for ensuring the purity of the seed stock were adequately described in the method of manufacture and quality assurance program.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The potency (CFU/g) of the technical grade active ingredient and the end-use products will be determined by direct counting using microscopy and plate counting on selective media.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

In the event it becomes required to analyse for residues of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 in plants, DNA isolated from single spore isolations and then the PCR methods developed to identify each of the MPCAs in section 2.1 could be used to analyse for each MPCA. The MPCAs are not known to produce any relevant metabolites.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The manufacturing process described was for commercial scale production and includes steps for quality assurance. The quality assurance procedures that will be used to limit contaminating microorganisms during manufacture of *A. pullulans* strain DSM 14940, *A. pullulans* strain DSM 14941 and Blossom Protect are acceptable.

During manufacturing, several approaches will be used to limit microbial contamination in the TGAIs and EP. These approaches will include frequent purity checks using microscopic techniques, and plating on selective agar media, sterilization of all equipment and media, and sanitization of recovery equipment.

The absence of human pathogens and below-threshold levels of contaminants were shown in the microbial screening of five production batches using pathogen-specific growth media. Microbe-specific screening methods for enteric bacteria/total coliforms, yeasts/moulds, Salmonella spp., and total mesophiles are adequate for detecting and enumerating microbial contaminants of concern. Release standards for microbial contaminants comply with those permitted by the PMRA and are adequate to ensure that the EP does not contain unacceptable levels of human and animal disease-causing microorganisms.

No known toxic metabolites or hazardous substances are present in Blossom Protect. Some strains of A. *pullulans* are known to produce secondary metabolites, aureobasidins, which act either as antifungal or antibacterial agents. However, *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 did not show any antibiotic activity.

2.6 Methods to Determine Storage Stability, Shelf-life of the Microorganism

Results from storage stability testing of three batches of the EP showed that it is stable when stored at 8°C for 2 years and at 25°C for 10 months.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

The PMRA conducted a detailed review of the toxicological database for the TGAIs, *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941, and the EP, Blossom Protect. The database is complete, consisting of laboratory animal (*in vivo*) toxicity studies (acute oral toxicity, acute pulmonary toxicity/pathogenicity, acute inhalation toxicity, acute subcutaneous infectivity, acute dermal toxicity/irritation, dermal sensitization, and eye irritation) that were carried out in accordance with currently accepted international testing protocols and Good Laboratory Practices. Additional genotoxicity testing was performed on mice. The scientific quality of the data is high and the database is considered sufficient to characterise the toxicity and infectivity of this pest control agent and product. See Table 1 in Appendix I for details.

In an acute oral toxicity study, four groups of Wistar SPF rats (3 ♀ / 3♂) of unknown age (subject weight ranges were ♂ 350 – 390 g and ♀ 220 – 260 g at dosing) were given a single oral dose of the MPCA Yeast Isolate *A. pullulans* strain DSM 14941 (4×10^8 colony forming units (CFU)/g) at doses of 1 g (dosing vehicle was not specified). The animals were then observed for a period of up to 21 days with interim sacrifices on Days 3, 7, and 14. Untreated, untreated shelf, and inactivated test substance controls were employed. Based on the results of this study, *A. pullulans* strain DSM 14941 is of low toxicity and does not appear to be pathogenic to the rat; however. There were no treatment related clinical signs, necropsy findings or changes in body weight. This acute oral study is classified as acceptable for toxicity only. This study satisfied the guideline requirement for an acute oral toxicity study in the rat; however, it did not satisfy the guideline requirement for an acute oral infectivity study in the rat. The microbial enumeration method was not validated and it failed to consistently detect the MPCA in sample tissue. A pattern of clearance of the MPCA from the rat was not determined.

In an acute oral toxicity study, two groups (3♀/group) of 8-week old Crl:CD(SD)IGS BR rats were given a single oral dose of an EP similar to Blossom Protect, BP2042 (2×10^{10} blastospores *A. pullulans* DSM 14940 per g, 7×10^9/g germinable; 2×10^{10} blastospores *A. pullulans* DSM 14941 per g, 7×10^9/g germinable), in deionized water at doses of 2000 mg/kg bodyweight. The animals were then observed for a period of up to 14 days. No control groups were employed. The oral LD_{50} for females was greater than 2000 mg/kg bw (8×10^{10} blastospores [nominal]/kg bw). Based on the results of this study, BP2042 (and subsequently *A. pullulans* DSM 14940 and *A. pullulans* DSM 14941) is of low toxicity. There were no treatment related clinical signs, necropsy findings or changes in body weight. This acute oral study is classified as acceptable.

In an acute pulmonary infectivity and toxicity study, groups of 205 – 310 g Wistar SPF rats (17 ♀, 17♂) were exposed by the intratracheal route to the MPCA, *A. pullulans* strain DSM 14941 (4×10^8 CFU/g) in 0.3 mL of water at a dose of 0.8×10^8 CFU/animal (0.2 g test substance). Animals were then observed for up to 21 days with interim sacrifices performed on Days 0, 3, 7 and 14. Untreated control and untreated shelf controls were employed. The pulmonary LD_{50} for rats was greater than 0.8×10^8 CFU/animal. There were no mortalities observed in this limit test. Based on these results, *A. pullulans* strain DSM 14941 is of low toxicity and is not infective or pathogenic in the rat. There were no treatment related clinical signs, necropsy findings or changes in body weight. Microbial enumeration of organ tissues from treated animals revealed that a pattern of clearance of the microbial pest control agent was achieved by Day 7. This acute pulmonary infectivity and toxicity study is classified as acceptable.

In an acute inhalation toxicity study, one group of 9-week old, Sprague Dawley, SPF rats (5 ♀, 5 ♂) were exposed by the inhalation route (nose-only) to 5.17 mg/L of a 10% suspension of BP2042 (2×10^{10} blastospores *A. pullulans* DSM 14940 per g, 7×10^{9}/g germinable; 2×10^{10} blastospores *A. pullulans* DSM 14941 per g, 7×10^{9}/g germinable) in distilled water for 4 hours (actual concentration of test material was 0.52 mg/L). Animals then were observed for 14 days. No control group was employed. Inhalation LC_{50} for males and females was greater than 0.52 mg/L. No mortalities, adverse in-life observations, or abnormalities during necropsy were reported. This acute inhalation study is classified as supplemental because the minimum concentration of 2 mg/L for a limit test was not achieved.

In an acute intravenous/subcutaneous infectivity study, groups of 9 – 10-week old Wistar rats (3 ♀; 3 ♂/group) were injected subcutaneously with either *A. pullulans* strain DSM 14940 (2.36×10^{10} CFU/g) or *A. pullulans* strain DSM 14941 (1.50×10^{10} CFU/g) in 1 mL of 0.9% saline solution at a dose of 1.95×10^{7} CFU/animal. Animals were then observed for up to 28 days with interim sacrifices performed on Days 1 and 7. Two negative control groups and one control group each dosed with either inactivated *A. pullulans* strain DSM 14940 or inactivated *A. pullulans* strain DSM 14941 were used. *Auerobasidium pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 are not pathogenic based on the results of the study. A pattern of clearance of the MPCA was established by Day 28. Some instances of inflammation were observed in the skin of treated animals at the site of administration but were found to be subsiding by the end of the study, however, it is likely due to foreign proteins rather than fungal infection since the effects were similar between the groups treated with inactivated and active *A. pullulans* strain DSM 14940 and strain DSM 14941. There were no other treatment-related clinical signs, necropsy findings or changes in body weight. There were no mortalities. This subcutaneous injection infectivity study is classified as acceptable.

In an acute subcutaneous infectivity study, groups of Wistar rats (3 ♀10-week old; 3 ♂ 7-week old/group) were injected subcutaneously with *A. pullulans* strain DSM 14941 (1.1×10^{9} CFU/g) in 1 mL of 0.9% saline solution at a dose of 1.6×10^{7} CFU/animal. Animals were then observed for up to 28 days with interim sacrifices performed on Days 1 and 7. Two negative control groups and one control group dosed with inactivated *A. pullulans* strain DSM 14941 were used. *Auerobasidium pullulans* strain DSM 14941 is not pathogenic based on the results of the study. A pattern of clearance of the MPCA was established by Day 28. Severe inflammation and purulent abscesses observed in the skin of the administration area are likely due to immunological reactions to foreign proteins rather than fungal infection since the effects were similar between the groups treated with inactivated and active MPCA. Furthermore, a microbial examination revealed that purulence from abscesses was sterile in most cases (one positive result for gram negative bacteria was observed). There were no other treatment related clinical signs, necropsy findings or changes in body weight. There were no mortalities. This subcutaneous injection infectivity study is classified as acceptable.

In an acute subcutaneous toxicity study, groups of fasted, 8-week old Crl:CD(SD)IGS BR rats (5 per sex) were given a single subcutaneous injection of BP2042 (2×10^{10} blastospores *A. pullulans* DSM 14940 per g, 7×10^{9}/g germinable; 2×10^{10} blastospores *A. pullulans* DSM 14941 per g, 7×10^{9}/g germinable) in the shoulder region. The injection was given in 10 mL/kg bw of distilled water at doses of 2000 mg per kg bw. The animals were then observed for a period of up to 14 days. The subcutaneous LD_{50} for male and female rats was greater than 2000 mg/kg bw. One male animal was euthanized on Day 5 for humane reason due to a ruptured abscess. All other animals survived for the duration of the study. Based on the results of this study, BP2042 is not pathogenic via the subcutaneous route. Shortly after administration and lasting until the scheduled end of the study a bladder caused by the injection material was present at the injection site. Dermal swelling was observed in all animals, and ruptured abscess in the area of injection was observed in 7 animals. These effects are likely the result of an immune reaction to the relatively large volume of foreign material administered. There was no indication for systematic distribution or growth of fungi (i.e. MPCA). This acute subcutaneous toxicity study is classified as acceptable.

In an acute dermal toxicity study, a group of 8-week old female and 12-week old male Crl:CD(SD)IGS BR rats (5/sex) were dermally exposed to BP2042 (2×10^{10} blastospores *A. pullulans* DSM 14940 per g, 7×10^{9}/g germinable; 2×10^{10} blastospores *A. pullulans* DSM 14941 per g, 7×10^{9}/g germinable) for 24 hours to an area of approximately 10% of body surface area. Following exposure, the animals were observed for a period of 14 days. Dermal LD_{50} for male and female rats was greater than 2000 mg/kg bw. Based on the results of this study, BP2042 is of low toxicity via the dermal route. This acute dermal toxicity study is classified as acceptable.

In a primary dermal irritation study, three female New Zealand White rabbits were dermally exposed to 0.5 g of BP2042 (2×10^{10} blastospores *A. pullulans* DSM 14940 per g, 7×10^{9}/g germinable; 2×10^{10} blastospores *A. pullulans* DSM 14941 per g, 7×10^{9}/g germinable) in 1.0 mL of deionized water for 4 hours to 2.5 cm × 2.5 cm of body surface area. Animals then were observed for 72 hours. Irritation was scored by the method of Draize. Skin appeared normal in all animals for the duration of the study. There was no irritation observed. In this study, BP2042 is not a dermal irritant to the skin of rabbits. This study is classified as acceptable.

In a skin sensitization study with BP2042 (2×10^{10} blastospores *A. pullulans* DSM 14940 per g, 7×10^{9}/g germinable; 2×10^{10} blastospores *A. pullulans* DSM 14941 per g, 7×10^{9}/g germinable) in white petrolatum, young adult Dunkin Hartley guinea pigs (20 ♀) were tested using the Buehler method. Twelve treated animals had positive skin reactions to the challenge administration. None of the naïve control animals had positive skin reactions to the challenge administration. In this study, BP2042 is a dermal sensitizer. This study satisfied the guideline requirement for a dermal sensitization study in the guinea pig.

In a primary eye irritation study, approximately 60 mg of BP2042 (2×10^{10} blastospores *A. pullulans* DSM 14940 per g, 7×10^{9}/g germinable; 2×10^{10} blastospores *A. pullulans* DSM 14941 per g, 7×10^{9}/g germinable) was instilled into the conjunctival sac of the right eye of three female New Zealand White rabbits. Animals were then observed for 72 hours. Irritation was scored by the method of Draize. One rabbit had mild conjunctival redness one hour after administration. No other effects were observed. In this study, BP2042 is not an irritant to the eye of the rabbit. This study is classified as acceptable.

Two groups of mice (5 ♀; 5♂/group) received single oral gavages of 2000 mg/kg body weight *A. pullulans* strain DSM 14941 in 10 mL water/kg body weight. One group of animals was sacrificed after 24 hours and the second group was sacrificed after 48 hours. Femoral bone marrow was harvested and analyzed. The ratio of polychromatic erythrocytes to total erythrocytes was determined, and 2000 immature erythrocytes/animal were scored for the incidence of micro-nucleated immature erythrocytes. No statistically significant increase in the number of micro-nucleated erythrocytes was observed compared to the negative control group. There was no indication of genotoxicity to mouse erythrocytes from the oral administration of *A. pullulans* strain DSM 14941.

Although not all required toxicity testing was conducted on each strain of MPCA individually, studies testing both strains simultaneously (i.e. studies using EP as the test substance) used doses that posed a maximum hazard for each strain. Other ingredients contained in the test substance used for these studies are not toxicologically significant and are not expected to have affected the results of the test. Therefore, no further toxicity data are required.

In published scientific literature (including a PubMed search using "Aureobasidium pullulans"), it was found that strains of *A. pullulans* can be an opportunistic pathogen in immunosuppressed individuals, for example, causing subcutaneous mycosis in a renal transplant patient through a contaminated catheter, keratitis in patients undergoing eye operations, and infection of lymphatic system in patient with erythema nodosum leprosum. All were treatable with amphotericin B. Also, *A. pullulans* was found in contaminated ventilation systems and humidifiers, and was cited as a cause of sensitization symptoms such as hypersensitivity pneumonitis (i.e. humidifier lung). There was also one report of an unusual skin infection caused by *A. pullulans* that started from a cat scratch in an immunocompetent individual that was treatable with amphotericin B. There were no reports of toxic metabolites produced by *A. pullulans*.

Aureobasidium pullulans is commercially used for the production of pullulan. Pullulan is a linear homopolysaccharide of glucose (α-(1→6) maltotriose) and has, together with its derivatives, demonstrated numerous uses in foods, pharmaceuticals, manufacturing, and electronics, for example, underivatised films readily dissolve in water, and thus melt in the mouth as edible food coatings. The physiological function of pullulan is uncertain. Since *Aureobasidium* species cannot catabolize pullulan significantly to metabolizable sugars, pullulan presumably does not serve as a storage material. It is generally believed that pullulan and similar polysaccharides serve to protect cells from desiccation or help them adhere to environmental substrates.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA, and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity tests.

Within the available scientific literature, there are no reports that suggest *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 have the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941.

3.2 Occupational / Bystander Exposure and Risk Assessment

3.2.1 Occupational

When handled according to the label instructions, the potential routes of handler exposure to Blossom Protect are pulmonary, dermal and to some extent ocular.

The potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, handlers and early-entry workers exists, with the primary source of exposure to workers being dermal. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. This MPCA has been identified as causing localized host immune responses when exposed to broken skin. Although there is one report of a skin infection in an immunocompetent individual following a cat scratch, there is no indication that it could penetrate intact skin of healthy individuals. In order to mitigate this hazard, applicators, mixer/loaders, and handlers will be required to wear coveralls and waterproof gloves in addition to shoes, and socks. Early-entry workers will be prohibited from entry into treated area until spray has dried unless wearing appropriate PPE, including water-proof gloves, long-sleeved shirt, long pants, and shoes plus socks.

Although the risk of toxicity is low in individuals exposed to large quantities of Blossom Protect, respiratory and dermal hypersensitivity could possibly develop upon repeated exposure to the product since the EP has been identified as a sensitizer. Specific label wording to minimize exposure to dusts or mists generated while handling or applying the product are required. Exposure in applicators, mixer/loaders, handlers, and early-entry workers will be mitigated by label statements requiring PPE noted in the previous paragraph as well as a dust/mist filtering respirator for applicators, mixer/loaders, and handlers.

In a primary eye irritation study, it was found that Blossom Protect was not irritating to the eyes. Eye goggles are not required for applicators, mixer/loaders, handlers or early-entry workers.

3.2.2 Bystander

Overall the Agency does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for Blossom Protect. As well, the active ingredients, *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941, belong to a species of yeast-like fungi that is ubiquitous in the environment and the use of Blossom Protect is not expected to increase exposure to bystanders beyond natural levels. Furthermore, since the use is agricultural, bystander exposure, including exposure to infants and children in schools, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to bystanders, including children and infants, is expected to be negligible.

3.3 Incident Reports Related to Human and Animal Health

Since April 26, 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Health Canada website. Incidents from Canada and the United States were searched and reviewed for products containing *A. pullulans*. As of April 26, 2012, there were no health-related incident reports submitted to the PMRA, nor summarised by the U.S. EPA or the California Department of Pesticide Regulation (CalDPR), for end-use products containing *A. pullulans*.

3.4 Dietary Exposure and Risk Assessment

3.4.1 Food

The applicant submitted published scientific literature that showed the population of *A. pullulans* was not significantly increased over naturally occurring densities after application of Blossom Protect to pome fruit. Therefore, an application of Blossom Protect during flowering is not expected to increase the natural colonisation density of *A. pullulans*.

While the proposed use pattern may result in some dietary exposure with possible residues in or on agricultural commodities, negligible to no risk is expected for the general population, including infants and children, or animals because *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity study. Furthermore, higher tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary and subcutaneous injection toxicity/infectivity studies. Therefore, there are no concerns for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.4.2 Drinking Water

No risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and because there were no harmful effects observed in Tier I acute oral toxicity testing and infectivity testing. The Blossom Protect label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Users are also requested to not allow effluent or runoff from greenhouses containing this product to enter lakes, streams, ponds or other waters. Furthermore, municipal treatment of drinking water is expected to remove the transfer of residues to drinking water. Therefore, potential exposure to *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 in surface and drinking water is negligible.

3.4.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e. no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the Agency concludes that the MPCAs are of low toxicity, are not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCAs than the general population. Thus there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCAs, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCAs and other registered micro-organisms that have a common mechanism of toxicity, does not apply to these MPCAs. As a result, the Agency has not used a margin of exposure (safety) approach to assess the risks of these MPCAs to human health.

3.5 Maximum Residue Limits

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the Pest Control Products Act (PCPA) for the purposes of the adulteration provision of the Food and Drugs Act (FDA). Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

Aureobasidium pullulans is ubiquitous in the phyllosphere and the application of Blossom Protect to pome fruit crops is not expected to significantly increase levels of *A. pullulans* beyond levels that have been observed in nature. There were no signs of toxicity and no signs of pathogenicity observed after the MPCAs were administered orally to rats and no metabolic by-product of toxicological concern are produced by these microorganisms. The establishment of a maximum residue limit (MRL) is therefore not required for *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 under Section 4(d) of the Food and Drugs Act (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulation.

3.6 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the Agency's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 to the general Canadian population, including infants and children, when the microbial pest control product is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Dermal and inhalation exposure to the general public will be very low since the product is to be used in agricultural sites and is not allowed for use on turf, residential or recreational areas. Furthermore, few adverse effects from exposure to other isolates of *A. pullulans* encountered in the environment have been reported. Even if there is an increase in exposure to this microorganism from the use of Blossom Protect, there should not be any increase in potential human health risk.

3.7 Cumulative Effects

The Agency has considered available information on the cumulative effects of residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *A. pullulans* in the environment, the Agency is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Aureobasidium pullulans is a yeast-like saprophytic fungal organism that is ubiquitously found in terrestrial ecosystems and has also been isolated from aquatic ecosystems. Levels of *Aureobasidium pullulans* isolated from apple leaves have been observed at levels up to 2.0×10^5 CFU/g.

Using the application rate of Blossom Protect to estimate populations of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 immediately after application results in a level comparable to natural background levels of *A. pullulans*. Therefore, an increase in population density of *A. pullulans* is not expected; however, an increase in the distribution of *A. pullulans* is expected. Since the growth of the microbial pest control agents (MPCAs) are dependent upon nutrient availability and environmental conditions, the background level of the MPCAs are expected to return to site specific background levels of *A. pullulans* over the growing season.

4.2 Effects on Non-Target Species

4.2.1 Effects on Terrestrial Organisms

Several studies were submitted to address the hazards of the MPCA to terrestrial non-target organisms. These studies included non-target avian species and terrestrial arthropods. See Table 2 in Appendix I for details.

The acute oral pathogenicity and toxicity study of *A. pullulans* strain DSM 14941 to Japanese quail (*Coturnix coturnix japonica*) was assessed over 30 days in accordance with U.S. EPA OPPTS Microbial Pesticide Test Guideline 885.4050, *Avian Oral, Tier I. Aureobasidium pullulans* strain DSM 14941 was administered to the birds by oral gavage at 2000, 1000, or 500 mg/kg bw (1.1×10^{10}, 5.1×10^{9}, 2.5×10^{9} CFU/kg bw) for 5 consecutive days. The 30-day acute oral LD_{50} was greater than 2000 mg/kg bw (1.1×10^{10} CFU/kg bw). There were no treatment related effects observed. There were no signs of pathogenicity or infectivity. This toxicity and pathogenicity study is classified as acceptable and satisfies the guideline requirement for an avian oral toxicity and pathogenicity study.

In a 22-day dietary toxicity/pathogenicity study, honeybees (*Apis mellifera*) were fed daily with Blossom Protect (Component B) in a 50% w/v sucrose and distilled water suspension at a rate of 200 µg test material/bee (1×10^{6} CFU/bee) in accordance with OECD Guidelines for the Testing of Chemicals 213 – *Honeybees, acute oral toxicity test*. The viability of microbial pest control product (MPCA) in the test substance was confirmed. Mortality was not significantly different between the test groups and there were no toxic symptoms observed in the test groups during the test period. There were no signs of infectivity or pathogenicity. The 22-day LC_{50} was greater than 200 µg (1×10^{6} CFU)/bee. This study is classified as acceptable and satisfies the guideline requirement for a dietary toxicity study for honey bees.

A summary paper of a study where bees were used to vector the MPCAs to apple and pear blossoms was submitted. Five colonies of bees were treated. Bees leaving the treated colonies passed through a dispenser containing the MPCAs contained in Blossom Protect. The quantity of MPCAs on bees after leaving the colony and upon return was measured at 10^{6} and 10^{5} CFU/bee respectively. No adverse effects to treated bee colonies were observed over a period of 23 days.

In a 14-day contact toxicity/pathogenicity study, predatory mites (*Typhlodromus pyri*) were exposed to dry residues of Blossom Protect that were applied to a hard surface (glass) in a spray concentration of 1.91×10^8 colony forming units (CFU)/mL in accordance with U.S. EPA OPPTS 885.4340 – *Nontarget insect testing, Tier I*. Mortality was observed until Day 7 and reproduction was observed from Days 7 to 14. There were no statistically significant differences in mortality or reproduction between the test group and negative and vehicle control. There were no signs of pathogenicity. Blossom Protect Component B is not toxic, and does not affect the reproduction of predatory mites when exposed for 14 days to dried residues of test concentrations containing 1.91×10^8 CFU/mL. This study is classified as acceptable and satisfies the guideline requirement for a contact toxicity study for terrestrial arthropods.

From the data submitted under the Part M4 Human Health and Safety Testing it was determined that *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 were not toxic to mammals via the oral, pulmonary or dermal routes, they were also not pathogenic via the pulmonary or subcutaneous route. No further data are required to assess the risk of harm to non-target wild mammals.

No toxicity/pathogenicity data were considered to address the potential for harm to non-target plants, terrestrial non-arthropod invertebrates or microorganisms. However, since these organisms are not expected to be exposed to significantly higher concentrations of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 compared to naturally occurring levels of *A. pullulans*, no further data are required to assess the risk of harm to non-target plants, terrestrial non-arthropod invertebrates or microorganisms.

Based on all the available data and information on the effects of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 to non-target terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, terrestrial arthropods, terrestrial non-arthropod invertebrates, terrestrial plants, and microorganisms from the proposed use of Blossom Protect.

4.2.2 Effects on Aquatic Organisms

Several studies were submitted to address the hazards of the MPCA to aquatic non-target organisms. These studies included non-target fish species, aquatic arthropods and aquatic plants. See Table 2 in Appendix I for details.

In a 96-hour toxicity study, rainbow trout (*Oncorhyncus mykiss*) were exposed to Component B of Blossom Protect under static/renewal conditions. Test fish were exposed aquatically to 100 mg test substance/L. The study was conducted in accordance with OECD Guideline for Testing of Chemicals 203 – *Fish acute toxicity test*. There were no mortalities, no signs of toxicity and no other treatment related effects observed. The 96-hour LC_{50} for rainbow trout was greater than 100 mg/L.

In a 21-day toxicity/pathogenicity study, daphnia (*Daphnia magna*) were exposed to Blossom Protect (Component B) under static renewal conditions. Daphnids were exposed aquatically to concentrations of 200, 100, and 50 mg test material/L (1.1×10^6, 5.3×10^5, 2.7×10^5 CFU/mL) and observed for mortality, reproduction, and body length. The study was conducted in accordance with U.S. EPA OPPTS 885.4240–*Freshwater aquatic invertebrate testing, tier I*. The mortality rate was 5% for the negative control and all test groups. The 200, 100, and 50 mg/L groups had reproductive rates that were 52.7, 34.0, and 53.1 % greater than the control group respectively and body lengths that were 43.2, 30.3, and 44.2% greater than the control group respectively. The increase in reproduction and body weight can be attributed to the daphnids using the test material as a food source. There were no signs of pathogenicity observed. The 21-day LC_{50}, EC_{50}, and LOEC for Blossom Protect (Component B) is > 200 mg test item/L (corresponding to > 1.3×10^6 CFU/mL) and the NOEC is 200 mg test item/L (1.3×10^6 CFU/mL). This study is classified as acceptable and satisfies the guideline requirement for a toxicity/pathogenicity study for aquatic arthropods.

The effect of Blossom Protect Component B on the freshwater floating aquatic vascular plant, duckweed (*Lemna gibba*), was studied during a 7-day exposure period at a nominal concentrations of 250 mg/L (measured at a concentration of 8.0×10^8 CFU/L) under static/renewal conditions in accordance with U.S. EPA OPPTS 885.4300 – *Nontarget plant studies, Tier I*. There were no adverse treatment related effects observed. The 7-day EC_{50}s and LOECs for growth rate (frond number and dry weight) and yield (frond number and dry weight) were all > 250 mg/L (8.0×10^8 CFU/L) and the respective NOECs were 250 mg/L (8.0×10^8 CFU/L). This study is classified as acceptable and satisfies the guideline requirement for an acute toxicity study for aquatic vascular plants.

No toxicity/pathogenicity data were considered to address the potential for harm to aquatic non-target non-arthropod invertebrates. However, since these organisms are not expected to be exposed to significantly higher concentrations of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 compared to naturally occurring levels of *A. pullulans*, no further data are required to assess the risk of harm to aquatic non-target non-arthropod invertebrates.

Based on all the available data and information on the effects of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 to non-target aquatic organisms, there is reasonable certainty that no harm will be caused to fish, aquatic arthropods, aquatic non-arthropod invertebrates or aquatic plants from the proposed use of Blossom Protect.

4.3 Incident Reports related to the Environment

Since April 26, 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website http://www.hc-sc.gc.ca/cps-spc/pest/part/protect-proteger/incident/indexeng.php. Only incidents in which the pesticide is determined to be linked to the effects (Canadian causality of highly probable, probable and possible; U.S. causality of highly probable, probable and possible) are considered in the reviews.

As of April 20, 2012, there were no environmental incidents reported in the PMRA Incident reporting database nor in the USEPA's Ecological Incident Information System (EIIS) for products containing *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 for use as pesticides, including the U.S. EPA registered product Biotector and Blossom Protect which contains the active ingredients *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941.

5.0 Value

5.1 Effectiveness Against Pests

5.1.1 Acceptable Efficacy Claims

Control of fire blight (*Erwinia amylovora*) in pome fruits and suppression of fire blight (*Erwinia amylovora*) in woody Rosaceae ornamentals

A total of 21 trials were submitted (18 on apples; 3 on pears) and reviewed to support the proposed claims. Disease pressure varied from low to high (4 to 64% disease incidence) in the efficacy trials. Reduction of disease incidence from Blossom Protect averaged 72% when applied 2 to 5 times at the proposed rate (0.75 kg a.i./ha/meter crown height) on apple and pear trees. Overall, Blossom Protect reduced the incidence of fire blight on apples and pears when applied according to the proposed use pattern and was statistically comparable to or better than other registered products for the same use in Canada including streptomycin sulphate, copper oxychloride, *Bacillus subtilis* and *Pantoea agglomerans*. A scientific rationale was provided by the applicant to extrapolate a claim for suppression of fire blight to woody Rosaseae ornamentals.

Based on efficacy data and scientific rationale, the claims of Blossom Protect to control fire blight in pome fruits and suppression of fire blight in woody Rosaceae ornamentals are supported according to the proposed use pattern.

5.2 Phytotoxicity to Host Plants

There is concern of russeting on sensitive cultivars of apples and pears with the use of Blossom Protect. A statistical analysis was performed to examine the russeting on apples and pears treated with Blossom Protect in which a dataset of 113 trials originating from Germany and Austria were analysed. In conclusion, no clear correlation could be made between the use of Blossom Protect and an increase in russeting. Blossom Protect had less influence on russeting than environmental conditions.

5.3 Economics

No market analysis was done for this submission.

5.4 Sustainability

5.4.1 Survey of Alternatives

Refer to Appendix I, Table 3 for a summary of the active ingredients currently registered for the same uses as Blossom Protect.

5.4.2 Compatibility with Current Management Practices Including Integrated Pest Management

Aureobasidium pullulans strains are sensitive to chemical fungicides. Therefore, tank mixing with other fungicides is not recommended.

5.4.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

Blossom Protect contains a mixture of two yeast strains of *Aureobasidium pullulans* that competes with the causal pathogen of fire blight for space and nutrients. There is a low risk for resistance development associated with the active ingredients because of their mode of action and antagonistic activity. The development of resistance to this product is not a concern at this time.

5.4.4 Contribution to Risk Reduction and Sustainability

Some copper-based fungicides and antibiotics (i.e. streptomycin) are currently registered for control of fire blight in apple and pear in Canada. However, their uses are now limited because of copper damage to foliage and fruit, and high risk for streptomycin resistance development. Several biological fungicides are also registered for suppression of fire blight. Blossom Protect, a product with low risk for resistance development, offers an additional tool to the Canadian growers for managing fire blight in pome fruits. In addition, access to reduced-risk biopesticides for management of fire blight is very limited in the ornamentals sector. Blossom Protect has been identified as an effective solution for both conventional and organic producers. Registration of Blossom Protect will help reduce the reliance on antibiotics for fire blight management and therefore contributes to resistance management, as well as pesticide risk reduction.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The Toxic Substances Management Policy (TSMP) is a federal government policy developed to provide direction on the management of substances of concern that are released into the environment. The TSMP calls for the virtual elimination of Track 1 substances [those that meet all four criteria outlined in the policy, i.e. persistent (in air, soil, water and/or sediment), bio-accumulative, primarily a result of human activity and toxic as defined by the *Canadian Environmental Protection Act*].

During the review process, Blossom Protect, *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 were assessed in accordance with the PMRA Regulatory Directive DIR99-03[5] and evaluated against the Track I criteria. The PMRA has reached the following conclusions:

- *Aureobasidium pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 do not meet the Track 1 criteria because the active ingredients are organisms and hence are not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.

6.2 Formulants and Contaminants of Health or Environmental Concern

During the review process, contaminants in the technical and formulants and contaminants in the end-use products are compared against the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* maintained in the *Canada Gazette*[6]. The list is used as described in the PMRA Notice of Intent NOI2005-01[7] and is based on existing policies and regulations including: DIR99-03; and DIR2006-02[8] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- Blossom Protect (EP), *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 (TGAIs) do not contain any other formulants or contaminants of environmental concern identified in the *Canada Gazette*.

The use of formulants in registered pest control products is assessed on an ongoing basis through PMRA formulant initiatives and DIR2006-02.

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[5] Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*

[6] *Canada Gazette*, Part II, Volume 139, Number 24, SI/2005-11-30) pages 2641-2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* and in the order amending this list in the *Canada Gazette*, Part II, Volume 142, Number 13, SI/2008-67 (2008-06-25) pages 1611-1613: *Part I Formulants of Health or Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions and Part 3 Contaminants of Health or Environmental Concern.*

[7] Notice of Intent NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Products Act*

[8] Regulatory Directive DIR2006-02, *PMRA Formulants Policy*

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for *A. pullulans* strain DSM 14940, *A. pullulans* strain DSM 14941 and Blossom Protect were deemed adequate to assess their potential human health and environmental risks. The TGAIs were characterised and the specifications of the EP were supported by the analyses of a sufficient number of batches. Storage stability data were sufficient to support a shelf life of two years when stored at 8°C and ten months when stored at 25°C.

7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information submitted in support of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 were determined to be sufficiently complete to permit a decision on registration. Spores of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 were not pathogenic or infective in the rat via subcutaneous injection exposure routes. Spores of *A. pullulans* strain DSM 14941 were not pathogenic or infective in the rat via the pulmonary route. An EP similar to Blossom Protect, BP2042, was of low toxicity to the rat via the oral, inhalation and dermal exposure routes, and was not irritating to the skin and eyes of rabbits. BP2042 was a sensitizer to guinea pigs. *Aureobasidium pullulans* strain DSM 14941 was not found to be genotoxic in a mouse erythrocyte micronucleus test.

When handled according to prescribed label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with the primary source of exposure to workers being dermal and to a lesser extent inhalation. Precautionary statements on the EP label and the wearing of PPE by workers will adequately mitigate the risks from exposure. While *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 are sensitizing agents, inhalation and dermal exposure is not a concern if the required dust/mist filtering respirator/mask and appropriate PPE stipulated on the EP label are worn by handlers and applicators. Furthermore, precautionary labelling will alert users of the sensitization hazard of the product.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is expected to be minimal since Blossom Protect will only be applied to agricultural pome fruit and rosaceaous ornamentals. The product is not to be applied to residential or recreational areas.

7.3 Environmental Risk

The environmental fate studies, non-target organism testing, scientific rationales and supporting published scientific literature submitted in support of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 were determined to be sufficiently complete to permit a decision on registration. The use of Blossom Protect containing *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 is not expected to pose a risk to birds, mammals, arthropods, fish, and plants when the directions for use on the label are followed. No other environmental fate studies or non-target organism studies are required to consider a decision on the registration of Blossom Protect for use against fire blight on agricultural pome fruit and nursery rosaceous ornamentals.

As a specific precaution, the Blossom Protect label instructs users to not contaminate irrigation or drinking water supplies or aquatic habitats by application of product, cleaning of equipment or disposal of wastes.

7.4 Value

Value information was provided to support the use of Blossom Protect to control fire blight in pome fruits and to suppress fire blight in woody Rosaceae ornamentals. Blossom Protect has been identified as a low priority for control of fire blight on apples and pears in the Canadian Grower Priority Database (CGPD). It offers an additional tool for Canadian growers for disease and resistance management.

A summary of the proposed and accepted uses for Blossom Protect is presented in Appendix I, Table 4.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Aureobasidium pullulans* DSM 14940, *Aureobasidium pullulans* DSM 14941 and Blossom Protect, containing the technical grade active ingredient *Aureobasidium pullulans* strain DSM 14940 and strain DSM 14941, to control fire blight in pome fruits and suppression of fire blight in woody Rosaceae ornamentals.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

a.i.	active ingredient
ADI	acceptable daily intake
ARD	acute reference dose
bw	bodyweight
CFU	colony forming unit
CGPD	Canadian Grower Priority Database
cm	centimetre
DNA	deoxyribonucleic acid
DSM	German Collection of Microorganisms and Cell Cultures (aka DSMZ)
DSM	German Collection of Microorganisms and Cell Cultures (aka DSMZ)
EC_{50}	median effect concentration
EP	end use product
FDA	*Food and Drugs Act*
g	gram
ITS	internal transcribed spacers
kg	kilogram
L	litre
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LD_{50}	median lethal dose
LOEC	lowest observable effect concentration
mg	milligram
mL	millilitre
MPCA	microbial pest control agent
MRL	maximum residue limit
NIOSH	National Institute for Occupational Safety and Health
NOEC	no observed effect concentration
°C	degree(s) Celsius
OPPTS	Office of Pollution Prevention and Toxic Substances
PCPA	*Pest Control Products Act*
PCR	Polymerase Chain Reaction
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
ppm	parts per million
RAPD	randomly amplified polymorphic DNA analysis
TGAI	technical grade of the active ingredient
TSMP	Toxic Substances Management Policy
US EPA	United States Environmental Protection Agency

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of A. pullulans strain DSM 14940, A. pullulans strain DSM 14941 and Blossom Protect

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference
Acute Toxicity/Infectivity of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941				
Acute Oral Toxicity	Rat – Wistar SPF Four groups of 6 rats (3/sex) dosed with 4×10^8 CFU *A. pullulans* strain DSM 14941 (MPCA); interim sacrifices (3/sex) on Days 3, 7, 14 3/sex untreated control, shelf control, and inactivated test substance control Animals were observed until Day 21 for clinical signs, mortality, and body weight. Necropsy was performed upon sacrifice	$LD_{50} > 4 \times 10^8$ CFU/animal	No mortalities or effect on bodyweight gain, and no clinical signs of treatment-related toxicity, infectivity or pathogenicity. No significant findings observed at necropsy. **LOW TOXICITY** **ACCEPTABLE FOR TOXICITY ONLY**	PMRA 2060642
Acute Pulmonary Toxicity and Infectivity	Rat – Wistar SPF Five groups (17/sex) were dosed intratracheally with *A. pullulans* strain DSM 14941 at 0.8×10^8/animal (MPCA) in water; interim sacrifices on Days 0, 3, 7, and 14 2/sex untreated control and untreated shelf control Animals were observed until Day 21 for clinical signs, mortality, and body weight. Necropsy was performed upon sacrifice including organ weight and microbial enumeration of tissues	$LC_{50} > 0.8 \times 10^8$ CFU/animal	No mortalities, treatment related clinical signs, necropsy findings or changes in body weight No treatment-related toxicity, infectivity or pathogenicity Organ tissues from treated animals revealed that a pattern of clearance of the microbial pest control agent was achieved by Day 7. **LOW TOXICITY, NOT INFECTIVE** **ACCEPTABLE**	PMRA 2060682

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference
Acute Subcutaneous Injection	Rat – Wistar Three groups (3/sex) dosed with 1.95×10^7 CFU/animal of *A. pullulans* strain DSM 14940; and three groups (3/sex) dosed with 1.95×10^7 CFU/animal of *A. pullulans* strain DSM 14941; doses were injected subcutaneously; interim sacrifices on Days 1 and 7; final sacrifice on Day 28 Inactivated test substance and negative control Observed body weight, clinical signs, necropsy, microbial enumeration, and histopathology	Not pathogenic	Some instances of inflammation observed in treated animals at administration site subsiding by end of study; likely due to foreign proteins rather than fungal infection since effects were similar between treated groups and inactivated groups A pattern of clearance was determined by Day 28; no signs of growth of MPCA observed in histopathology No other clinical signs, mortalities, body weight changes or abnormal necropsy observations **NOT PATHOGENIC** **ACCEPTABLE**	PMRA 2060705
Acute Subcutaneous Injection Infectivity	Rat – Wistar Three groups (3/sex) dosed with 1.6×10^7 CFU/animal of *A. pullulans* strain DSM 14941; doses were injected subcutaneously; interim sacrifices on Days 1 and 7; final sacrifice on Day 28 Inactivated test substance and negative control Observed body weight, clinical signs, necropsy, microbial enumeration, and histopathology	Not pathogenic	Instances of inflammation and purulent abscesses observed in treated animals at administration site subsiding by end of study; likely due to foreign proteins rather than fungal infection since effects were similar between treated groups and inactivated groups A pattern of clearance was determined by Day 28; no signs of growth of MPCA observed in histopathology No other clinical signs, mortalities, body weight changes or abnormal necropsy observations **NOT PATHOGENIC** **ACCEPTABLE**	PMRA 2060706

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference
Mammalian Erythrocyte Micronucleus Test	Mouse Two groups (5/sex) received single oral gavages of 2000 mg/kg body weight *A. pullulans* strain DSM 14941 in 10 mL water/kg bw; interim sacrifice at 24 hours; final sacrifice at 48 hours Femoral bone marrow harvested and analyzed; ratio of polychromatic erythrocytes to total erythrocytes was determined; and 2000 immature erythrocytes/animal scored for the incidence of micro-nucleated immature erythrocytes.	No indication of genotoxicity to mouse erythrocytes	No statistically significant increase in the number of micro-nucleated erythrocytes was observed compared to the negative control group. No indication of genotoxicity to mouse erythrocytes from the oral administration	PMRA 2060753
Acute Toxicity/ Infectivity of BP2042 [similar to Blossom Protect] (2×10^{10} blastospores *A. pullulans* DSM 14940 per g; 2×10^{10} blastospores *A. pullulans* DSM 14941 per g)				
Acute Oral Toxicity	Rat - Crl:CD(SD)IGS BR Two groups (3 females/group) rats dosed with BP2042 at 2000 mg/kg body weight (bw) Observed for clinical signs, bw, and mortality until sacrifice and necropsy on Day 14	$LD_{50} > 2000$ mg/kg bw	No treatment related clinical signs, necropsy findings or changes in body weight. No mortalities. **LOW TOXICITY** **ACCEPTABLE**	PMRA 2060198
Acute Inhalation Toxicity	Rat – Sprague-Dawley One group (5/sex) exposed to BP2042 suspended in water at a concentration of 0.52 mg/L for 4 hours nose-only Observed mortality and clinical signs for 14 days Necropsy performed at sacrifice	$LC_{50} > 0.52$ mg/L	No mortalities, clinical signs, or abnormalities during necropsy **SUPPLEMENTAL**	PMRA 2060201

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference
Acute Subcutaneous Injection Toxicity	Rat – Crl:CD(SD)IGS BR One group (5/sex) given subcutaneous injections of BP2042 at doses of 2000 mg/kg bw; no control groups Clinical observations made daily for 14 days; body weight on Days 7 and 14; necropsy upon sacrifice on Day 14	LD_{50} > 2000 mg/kg bw	One male animal was euthanized on Day 5 due to a ruptured abscess. All other animals survived to Day 14. Dermal swelling was observed in all animals, and ruptured abscess in the area of injection was observed in 7 animals; likely the result of an immune reaction to the relatively large volume of foreign material administered. No indication for systematic distribution or growth of fungi and no signs of toxicity **LOW TOXICITY** **ACCEPTABLE**	PMRA 2060180 2060698
Acute Dermal Toxicity	Rat – Crl:CD(SD)IGS BR One group (5/sex) were dermally exposed to 2000 mg/kg bw BP2042 for 24 hours to an area of 10% of body surface. Observed for 14 days.	LD_{50} > 2000 mg/kg bw	No treatment related effects were observed. There were no mortalities. **LOW TOXICITY** **ACCEPTABLE**	PMRA 2060199
Dermal Irritation	Rabbit – New Zealand White Three females were dermally exposed to 0.5 g of BP2042 in 1.0 mL of deionized water for 4 hours to 2.5 cm × 2.5 cm area Observed for 72 hours. Irritation scored by method of Draize	Skin appeared normal	No irritation observed **NOT IRRITATING** **ACCEPTABLE**	PMRA 2060203
Eye Irritation	Rabbit – New Zealand white Three females were instilled with 60mg of BP2042 into the conjunctival sac of the right eye; rabbits were observed for 72 hours and irritation scored by the method of Draize	One instance of mild conjunctivitis	One rabbit had mild conjunctival redness one hour after administration **NOT IRRITATING** **ACCEPTABLE**	PMRA 2060204

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference
Dermal Sensitization	Guinea Pig – Dunkin Hartley 20 ♀ induced and challenged with BP2042 Induction: Days 0, 6, and 14 – topical application of 0.6 g in white petrolatum Challenge: Topical application of 0.5 g in white petrolatum on Day 27; scored on Days 28 and 29	Positive	Twelve treated animals had positive skin reactions to the challenge administration. **SENSITIZER** **ACCEPTABLE**	PMRA 2060205

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
		Vertebrates		
Birds	*Coturnix coturnix japonica* 30-day oral	Three groups of birds (10/group) were gavaged with 2000, 1000, or 500 mg of *A. pullulans* DSM 14941 (1.1×10^{10}, 5.1×10^{9}, or 2.5×10^{9} CFU) /kg bw for 5 consecutive days. One negative control (10 birds) was dosed with distilled water. Birds were observed for 30 days.	No treatment related mortalities or overt signs of toxicity were reported in the treatment groups. There were no signs of pathogenicity or infectivity. 30-day acute oral LD_{50} >2000 mg/kg bw **ACCEPTABLE**	PMRA 2060633
	Pulmonary	A request to waive the requirement for test data was not submitted. However, no toxicity or pathogenicity was observed in the avian acute oral toxicity study, therefore, the need for avian pulmonary testing has been waived.		
Wild Mammals	From the data submitted under the Part M4 Human Health and Safety Testing it was determined that *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 were not toxic to mammals via the oral, pulmonary or dermal routes, they were also not pathogenic via the pulmonary or subcutaneous route. No further data are required to assess the risk of harm to non-target wild mammals.			

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Invertebrates				
Arthropods				
Terrestrial Arthropods	*Apis mellifera* 22-day dietary	Three replicates (10 bees/ replicate) were fed daily with Blossom Protect (Component B) in a 50% w/v sucrose/distilled water suspension at 200 µg test material/bee (1×10^6 CFU/bee) for 22 days. One negative control with three replicates of 10 bees.	Test group mortality was not significantly different from control. There were signs of toxicity or pathogenicity. The 22-day LC_{50} was greater than 200 µg (1×10^6 CFU)/bee. **ACCEPTABLE**	PMRA 2060137
	Typhlodromus pyri 14-day contact	Three replicates of 20 predatory mites were exposed to dry residues of Blossom Protect Component B applied to glass in a spray concentration of 1.91×10^8 CFU/mL Vehicle and negative controls with 3 replicates of 20 mites each. Observed for mortality until Day 7 and reproduction for Days 7 to 14.	There were no statistically significant differences in mortality or reproduction between the test group and negative and vehicle controls. There were no signs of toxicity or pathogenicity. **ACCEPTABLE**	PMRA 2060128
Non-arthropods				
Terrestrial Non-Arthropod Invertebrates	A request to waive the requirement for test data was not submitted. However, since these organisms are not expected to be exposed to significantly higher concentrations of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 compared to naturally occurring levels of *A. pullulans*, no further data are required to assess the risk of harm to non-target terrestrial non-arthropod invertebrates.			
Plants				
Plants	A request to waive the requirement for test data was not submitted. However, since these organisms are not expected to be exposed to significantly higher concentrations of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 compared to naturally occurring levels of *A. pullulans*, no further data are required to assess the risk of harm to non-target terrestrial plants.			
Microorganisms				
Micro-organisms	A request to waive the requirement for test data was not submitted. However, since these organisms are not expected to be exposed to significantly higher concentrations of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 compared to naturally occurring levels of *A. pullulans*, no further data are required to assess the risk of harm to non-target microorganisms.			

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Aquatic Organisms				
		Vertebrates		
Fish	*Oncorhynchus mykiss* 4-day static renewal	Two replicates of 15 fish were exposed to 100 mg/L Blossom Protect under static renewal conditions. One negative control group (2 replicates of 15) held in untreated test water. Daily observations for mortality.	There were no mortalities, no signs of toxicity and no other treatment related effects observed. The 96-hour LC_{50} for rainbow trout was greater than 100 mg/L.	PMRA 2060133
		Invertebrates		
Aquatic Arthropods	*Daphnia magna* 21-day static renewal	Three groups (20/group) of daphnia were exposed to 200, 100, or 50 mg Blossom Protect (1.1×10^6, 5.3×10^5, 2.7×10^5 CFU/mL) /L for 21 days under static/renewal conditions. Negative control with 20 daphnids in untreated test culture. Observed for mortality, reproduction, and body length for 21 days.	Mortality rate of 5% after 21 days for all test groups and control. The 200, 100, and 50 mg/L groups had reproductive rates that were 52.7, 34.0, and 53.1% greater than the control group, respectively, and body lengths that were 43.2, 30.3, and 44.2% greater than the control group respectively. Increase in reproduction and body weight can be attributed to the use of test material as a food source. There were no signs of pathogenicity observed. The 21-day LC_{50} was greater than 200 mg/L (1.1×10^6 CFU/mL) **ACCEPTABLE**	PMRA 2060135
Aquatic Non-Arthropod Invertebrates	A request to waive the requirement for test data was not submitted. However, since these organisms are not expected to be exposed to significantly higher concentrations of *A. pullulans* strain DSM 14940 and *A. pullulans* strain DSM 14941 compared to naturally occurring levels of *A. pullulans*, no further data are required to assess the risk of harm to non-target aquatic non-arthropod invertebrates.			

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Plants				
Aquatic Plants	*Lemna gibba* 7-day static/renewal	Duckweed was exposed to 250 mg Blossom Protect / L under static/renewal conditions for 7 days. A negative control was tested in untreated test water. Frond number was observed on Day 0, 3, 5 and 7. Dry weight was measured on Day 7.	There were no adverse treatment related effects observed. The 7-day EC_{50}s, NOECs, and LOECs for growth rate (frond number and dry weight) and yield (frond number and dry weight) were all > 250 mg/L (8.0×10^8 CFU/L).	PMRA 2060141

Table 3 Summary of Alternatives for the Same Uses as BAS 700 01F and BAS 700 04F Fungicides

Disease	Active ingredient and FRAC Fungicide Group	Target crop and level of control
Fire blight (*Erwinia amylovora*)	*Bacillus subtilis* strain QST 713	Suppression on pome fruits
	Copper sulphate (M)	Control on apple and pear
	Copper oxychloride (M)	Control on apple, pear and quince
	Pantoea agglomerans strain C9-1	Suppression on apple, pear and non-bearing pome fruit nursery stock
	Pantoea agglomerans, strain E325	Suppression on apple, pear and non-bearing pome fruit nursery stock
	Pseudomonas fluorescens strain A506	Suppression on apple and pear
	Streptomycin sulphate	Control on apple and pear

Table 4 Use (label) Claims Proposed by Applicant and Accepted

Proposed claim	Accepted claim
Control of fire blight (*Erwinia amylovora*)/ pome fruit (bearing and non-bearing)/ Apply spray mixture as at a rate of 500L/ha per m crown height. For trees of 2 m crown height use 1.5 kg Component B together with 10.5 kg in 1000L water. For larger trees, adapt the application rate accordingly. According to phenology: apply up to 4 times at 10%, 40%, 70% and 90% open blossoms (BBCH 61-69). According to a forecast system (for example, Maryblyt): Apply a maximum number of 5 times when model indicates risk of infection. Application preferably in the evening. The solution should be stirred during application.	Accepted as proposed with label changes
Suppression of fire blight (*Erwinia amylovora*)/ woody Rosaceae ornamentals/use pattern as in pome fruit	Accepted as proposed

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A. List of Studies/Information Submitted by Registrant

1.0 Chemistry

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2060253 2011, Storage stability Blossom Protect Component B at 20 C, DACO: Document K,IIIM 2.2,M2.11 CBI

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B. Additional Information Considered

i) Published Information

1.0 Chemistry

2184963 Association between sensitization to *Aureobasidium pullulans* (*Pullularia sp*) and severity of asthma, DACO: M2.7.2

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